

containing aqueous medium in which the acid is present in the internal and external liposome phases; or

- (2) an aqueous medium containing a base which is substantially impermeable through the vesicle to give an basic liposome-containing aqueous medium in which the base is present in the internal and external liposome phases;

(b) adding:

- (1) to the thus-obtained acid liposome-containing aqueous medium a permanently charged, chargeable, or pH titratable chemical species which is a cationic chemical species, or
- (2) to the thus-obtained acid liposome-containing aqueous medium a permanently charged, chargeable, or pH titratable chemical species which is an anionic chemical species; and

(c) adding to the external liposome phase:

- (1) a base to provide a pH gradient across the membrane of the liposome and thereby induce the cationic chemical species to pass into the liposomes' internal acidic aqueous phase, or
- (2) an acid to provide a pH gradient across the membrane of the liposome and thereby induce the anionic chemical species to pass into the liposomes' internal basic aqueous phase;

wherein said cationic chemical species or said anionic chemical species is accumulated and entrapped within said liposome to produce a stable liposome vesicle-entrapped chemical

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animal

species, said stability being independent of maintenance of a pH gradient across the liposome membrane after entrapment of the chemical species such that after administration to an animal the chemical species is carried to its destination by the liposome vesicle before significant leakage occurs, and the animal suffers no long-term effects of the administration.

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47. The method according to Claim 46 wherein the chemical species is a drug.
48. The method according to Claim 46 wherein the aqueous medium is a buffer solution.
49. A liposome vesicle-entrapped chemical species prepared by the method of Claim 46.
50. A pharmaceutical preparation for administration in vivo to an animal comprising the liposome vesicle-entrapped chemical species according to Claim 49.
51. A pharmaceutical preparation for parenteral administration in vivo to an animal comprising the liposome vesicle-entrapped chemical species according to Claim 49, wherein the buffer has an osmolarity within the physiological range of an animal, the vesicles are suspended for administration in a bulk solution, and the bulk solution has a pH which is physiologically benign.

52. (Twice Amended) A method of preparing a stable liposome vesicle-entrapped chemical species, which method comprises:

(a) forming liposomes in:

- (1) an aqueous medium containing an acid which is substantially impermeable through the vesicle to give an acidic liposome-containing aqueous medium in which the acid is present in the internal and external liposome phases; or
- (2) an aqueous medium containing a base which is substantially impermeable through the vesicle to give a basic liposome-containing aqueous medium in which the base is present in the internal and external liposome phases;

(b) adding:

- (1) to the thus-obtained acid liposome-containing aqueous medium a permanently charged, chargeable, or pH titratable chemical species which is a cationic chemical species, or
- (2) to the thus-obtained acid liposome-containing aqueous medium a permanently charged, chargeable, or pH titratable chemical species which is an anionic chemical species; and

(c) adding to the external liposome phase:

- (1) a base in an amount effective to create a pH gradient between the external liposome phase and the internal liposome phase to

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thereby induce the cationic chemical species to pass into the liposomes' internal acidic aqueous phase, or

- (2) an acid in an amount effective to create a pH gradient between the external liposome phase and the internal liposome phase to thereby induce the anionic chemical species to pass into the liposomes' internal basic aqueous phase;

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cont wherein said cationic chemical species or said anionic chemical species is accumulated and entrapped within said liposome to produce a stable liposome vesicle-entrapped chemical species, said stability being independent of maintenance of the pH gradient after entrapment of the chemical species such that after administration to an animal the chemical species is carried to its destination by the liposome vesicle before significant leakage occurs, and the animal suffers no long-term effects of the administration.

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53. The method according to Claim 52 wherein the chemical species is a drug.

54. The method of Claim 52, wherein the charged chemical species is a drug having hydrophobic ions.

55. The method of Claim 54, wherein the drug having hydrophobic ions is ellipticinium chloride, an antihelminthic, gentian violet, pyrvinium, pamoate, a cyanine dye, or pamaguine.

56. The method of Claim 53, wherein the drug is a drug for chemotherapy or immunosuppression, a membrane permeable peptide toxin or a hormone.

57. The method of Claim 52, wherein the pH titratable chemical species is a drug having molecules with basic properties.

58. The method of Claim 57, wherein the drug is vincristine, doxorubicin, streptomycin, chloroquine, daunorubicin.

59. The method of Claim 52, wherein the pH titratable chemical species is a drug having molecules with acidic properties.

60. The method of Claim 59, wherein the drug is a derivative of methotrexate, daunomycin, penicillin or a salicylic acid derivative.

61. The method according to Claim 52 wherein the aqueous medium is a buffer solution.

62. A liposome vesicle-entrapped chemical species prepared by the method of Claim 52.

63. A pharmaceutical preparation for administration in vivo to an animal comprising the liposome vesicle-entrapped chemical species according to Claim 62.

64. A pharmaceutical preparation for parenteral administration in vivo to an animal comprising the liposome vesicle-entrapped chemical species according to Claim 62, wherein the buffer has an osmolarity within the physiological range of an animal, the vesicles are suspended for administration in a bulk solution, and the bulk solution has a pH which is physiologically benign.

65. The method of Claim 60, wherein said salicylic acid derivative is p-amino salicylic acid.